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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : C12P 7/64, A23L 1/035, A23D 9/00

A1

(11) International Publication Number: **WO 91/06661**

(43) International Publication Date: 16 May 1991 (16.05.91)

(21) International Application Number: PCT/US90/06495

(22) International Filing Date: 2 November 1990 (02.11.90)

(30) Priority data:
431,567 3 November 1989 (03.11.89) US

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(81) Designated States: AT (European patent), BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent).

Published
With international search report.

(54) Title: LIPASE-CATALYZED IN SITU GENERATION OF MONO- AND DI-GLYCERIDES

(57) Abstract

Enzymatic modification of fats and oils results in a food-grade product that can be used as an emulsifier and/or fat replacer in foods. The modified oil is a mixture of mono-, di- and triglycerides having emulsification properties which can be added to commercial food preparations to replace all or part of the oil or fat in the food preparation.

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LIPASE-CATALYZED IN SITU GENERATION OF MONO- AND DI-GLYCERIDES

Background

Edible oils are widely used in the food industry. They have a number of properties that make them indispensable in a variety of applications. For example, fats and oils represent an important source of energy. They are also crucial in controlling the texture of such products as cakes, biscuits, pastries, and margarines by providing the desired texture, mouthfeel and other organoleptic properties. In most foods the addition of fats is accompanied by the addition of an emulsifier. Emulsifiers reduce the interfacial tension between the two immiscible liquids, water and oil, and allow the formation of a more stable and uniform homogeneous dispersion.

Emulsifiers are also useful in the production of low-calorie foods. A certain portion of the fat in a food can be substituted with a suspension of an emulsifier in water which results in a product with fewer calories. In most applications, a mixture of an oil and a synthetic, chemically produced emulsifier is added to foods, which reduces the value of a number of foods since they cannot be labeled "natural products".

An alternative way to introduce an emulsifier into a product without compromising its "naturalness" is to use a naturally-modified food grade oil that has emulsifying properties. For

example, it has been reported that a vegetable oil can be fermented with an organism to produce a vegetable oil product containing an emulsifier. R.D. Scwartz et al., U.S. Patent Number 4,810,507. The resultant fermented vegetable oil reduces the surface tension of an oil-water emulsion and can be used as an emulsifying agent in the food industry. However, this preparation contains yeast organisms and other impurities.

A natural, edible oil having emulsification properties is needed for use in the food industry.

Summary of the Invention

The invention relates to a method of preparing a food grade oil-based product with emulsifying and fat-sparing characteristics. The product is a mixture of an oil and mono- and di-glycerides formed by enzymatic partial hydrolysis or transesterification of the oil. The product can be used to replace fats and oils in a variety of food products and does not require the addition of an emulsifier.

In the present method, a selected lipase is combined with an oil and a certain amount of water or an aqueous solution or an alcohol, and the mixture is rapidly stirred to form a suspension. This suspension is agitated until the desired degree of hydrolysis (or transesterification, if an alcohol is added) of the oil is achieved. The lipase enzyme is then removed and the fatty-acid or fatty-acid ester by-products are separated from the

reaction mixture. The product is modified oil, which is a mixture of mono-, di- and triglycerides, having physical characteristics which are superior to that of the starting material.

The present process is free of organic solvent and no glycerol is formed during the reaction. The modified oil produced by the present method can be used successfully in a variety of food applications. Higher quality food products are obtained using the modified oil than non-modified oil due to the good emulsifying characteristics of the modified oil. In addition, due to their fat-sparing capabilities, that is, the ability to quantitatively replace the fats in a formulation, modified oils can be used as a fat replacement to obtain low-calorie food products. Important properties of the modified oils, such as emulsifying activity, rheology and melting point, can be modified by varying the degree of hydrolysis or transesterification of the oil.

Detailed Description of the Invention

In the present method, modified oils are produced in situ via lipase-catalyzed partial hydrolysis of oils in an organic solvent-free system, or by partial transesterification of oils in a system containing a small amount of alcohol. The method involves combining the oil, a small amount of water or an aqueous solution or an alcohol, and a selected lipase catalyst and stirring the mixture to form a suspension. The lipase partially hydrolyzes

the oil, or transesterifies it if an alcohol is added, forming mono- and di-glycerides. The resulting product is a mixture of the unreacted oil, and mono- and di-glycerides.

Any edible fat or oil or combination of fats or oils, can be used as the starting material in this process. Oils and fats which are useful include, for example, oils such as canola, soybean, sunflower, corn, olive, peanut, safflower, hydrogenated vegetable oil and other vegetable and animal oils, or fats such as butter fat and cocoa butter.

Lipases obtained from a variety of souces, including mammals, yeast, mold and bacteria can be employed as the catalyst in the present process. Lipases used in this process should exhibit high operational stability (e.g., can be reused without a significant loss of activity for at least 50 hours), be active at low water activity and efficiently catalyze the hydrolysis or transesterification of long chain (C_{12}-C_{18}) triglycerides. Lipases which are particularly useful in the present process are, for example, lipases derived from porcine pancrease, and from Pseudomonas fluorescence, Aspergillus niger, Mucor meihei, and Rhizopus niveus.

The reaction is carried out in the oil medium. A small amount of water or an aqueous solution or an alcohol, for example, from about 2% to about 8% by weight of the oil, is added to the oil and a selected lipase, preferably immobilized on an appropriate carrier (e.g., silica, diatomaceous earth, polystyrene), is added to the reaction

mixture. The amount of lipase added will vary depending on the enzymatic activity of the enzyme. Generally, from about 1 to about 5 mg/mL of lipase is added. Immobilized enzymes are particularly useful as they are easy to remove from the reaction mixture. Water can be added, or a water-based solution such as an aqueous salt solution. Aqueous solutions which can be used include, for example, salt solutions having a salt concentration of from about 1 mM to about 50 mM. Particularly useful salt solutions include, for example, 20 mM $CaCl_2$ or $MgCl_2$. Alcohols which can be used are primary alcohols such as ethanol, propanol and butanol.

The reaction can be carried out in any appropriate vessel, including a stirred tank reactor, or a packed column. If a tank reactor is used, the reaction mixture should be agitated by shaking or stirring. Agitation speed should be sufficient to form and maintain the suspension.

The temperature of the reaction mixture can range from about 20°C to about 60°C. A preferred temperature range is from about 25°C to about 45°C.

The reaction should be allowed to proceed for a time sufficient to produce about 2% by weight or higher, of monoglycerides. Reaction time can vary from about 2 to about 24 hours depending on the amount and activity of the catalyst. The course of the reaction can be monitored by chromatography (e.g., gas or thin layer chromatography). After the reaction is complete, the immobilized lipase is removed, for example, by centrifugation or by

filtration. The by-products of the reaction, fatty acids, or fatty acid esters, are then separated from the reaction mixture. This can be accomplished, for example, by ion-exchange chromatography, extraction or distillation.

The present method is simple, quick and results in a pure product which contains no organic solvents, free fatty acids or fatty acid esters, glycerol or catalyst. The product contains about 30 to about 60% by weight of the oil, and from about 70 to about 40% by weight mono- and diglycerides. The ratio of mono-, di- and triglycerides can be changed by changing the reaction time. Longer reaction times results in the formation of more mono- and diglycerides.

Modified oils prepared according to the present method are efficient emulsifiers and can be used to partially or totally replace the oils used in commercial food preparations. An advantage of the modified oils resides in the inherent emulsifying properties, whereas regular non-modified oils require the addition of emulsifiers. The modified oils can also be used in other applications such as formulating lotions or cream cosmetics and/or as a stabiler or thickener in foods or cosmetics. Modified oils produced by the present method are natural, contain no synthetic components or additives, and are substantially free of fatty acids or fatty esters, and glycerol.

The invention is further illustrated by the following exemplification:

EXEMPLIFICATION

MATERIALS

Lipases (EC 3.1.1.3) were obtained from the following suppliers: porcine pancreatic lipase (1.1 IU/mg solid) from Sigma Chemical Co. (St. Louis, MO) and Pseudomonas fluorescence (30 IU/mg solid) Aspergillus niger, Mucor meihei, and Rhizopus niveus from Amano International Enzyme Co. (Troy, VA). "Crisco" brand pure vegetable oil (The Procter and Gamble Company, Cincinnati, OH) and "Hollywood" brand peanut oil (Hollywood Foods, Los Angeles, CA) were purchased in a local supermarket. Canola oil and partly hydrogenated canola oil were purchased from Polyester Corporation (Southampton, NY) and CSP Foods LTD (Dundas, Ontario), respectively. Hexamethyldisilazane (HMDS) and trimethylchlorosilane (TMCS) used for analysis by gas chromatography were obtained from Pierce Co. (Rockford, IL). Amberlite XAD-7 used for lipase immobilization, and anion exchange resin AG 1-X8, 20-50 mesh in hydroxide form, used for removal of fatty acids, were purchased from Aldrich Chemical Company (Milwaukee, WI) and Bio-Rad Laboratories (Richmond, CA), respectively.

METHODS

Lipase Assays

The activity of the lipases in the hydrolysis reaction was determined potentiometrically using the

Radiometer RTS-812 recording pH-stat system (Rainin Instrument Co., Inc., Woburn, MA) using vegetable oil as a substrate. In this procedure, 10 mL of a 0.1 g/mL aqueous emulsion of a substrate containing 20 mM $CaCl_2$ was placed in the cuvette of the pH-stat, and the pH was adjusted to 7.5. Lipase was then added, and the acid liberated as the result of hydrolysis was automatically titrated with 0.1 M NaOH.

All products of enzymatic conversions were assayed by gas chromatography (Hewlett Packard 5890A) using 12-m fused silica capillary column (S.G.E. Australia). Nitrogen was used as a carrier gas (5 mL/min). Detector and injector port temperature were 350°C. Prior to injection, the samples were modified with hexamethyldisilazane following the standard procedure of Sweely et al.. Sweely et al., (1963), J. Am. Chem. Soc., 85:2495-2507.

In addition to gas chromatography, the course of the reaction was followed and the purity of all products were analyzed by thin-layer chromatography (TLC) using Whatman K6 silica gel sheets. A mixture of petroleum ether (b.p. 30-60°C), ether and acetic acid in a ratio of 70:30:1 was used as an eluting buffer. The spots were developed with iodine vapor.

Evaluation of Modified Vegetable Oils (MVO)

The effect of modification of vegetable oils on emulsifying properties was evaluated by preparing an emulsion of oil in water and recording the elapsed time for separation of the emulsion at room

temperature. Wesson brand oil was used as a control.

Using a high shear mixer, 20 grams of Wesson brand oil or the MVO to be tested were mixed with 80 grams of water. The mixture was then homogenized using a hand homogenizer and placed in a 25 mL graduated cylinder at room temperature. The level of oil separation as a function of time was recorded.

Performance in Yellow Cake

MVO was used as an ingredient in a yellow cake formulation replacing the partly hydrogenated oil and liquid vegetable oil. The following formulation and preparation procedures were used:

COMPOSITION AND PREPARATION PROCEDURE OF YELLOW CAKE WITH AND WITHOUT MVO*

Ingredients	Percent (by weight)		
	1	2	3
Water	29.25	29.25	29.25
Granulated sugar	26.80	26.80	26.80
Cake flour	25.40	25.40	25.40
Partially Hydrogenated Oil (Creamtex)	12.00	--	--
Whole egg powder	3.50	3.50	3.50
Baking powder	1.20	1.20	1.20
Whole milk powder	0.65	0.65	0.65
Vanilla extract	0.60	0.60	0.60
Salt	0.60	0.60	0.60

Vegetable Oil (Wesson)	--	12.00	--
MVO	--	--	12.00

Preparation Procedure:

1. Sugar, salt, milk powder, cake flour and oil or MVO were creamed, and 50% of the water added. The mixture was mixed for five to six minutes on medium speed.
2. 5% of water was added and mix for three minutes.
3. 1/2 of the egg powder, the remaining water, and the vanilla was added, and mixed on medium speed for four minutes.
4. Baking powder and egg powder were added and mixed for four more minutes.
5. 150 grams of the mixture was baked in 3" x 5" pan at 350°F for 22-23 minutes.

*MVO = modified vegetable oil

Source: ABIC International Consultants, Inc. (Fairfield, NJ)

Performance of Modified Vegetable Oil (MVO) in Tub Margarine

MVO was used as an ingredient in 80% and 40% fat tub margarine formulations. The following formulations were used:

80% Fat Margarine

Ingredients	Percent (by weight) 1	2
Oil Phase		
Margarine tub oil (Cargill 270)	79	77.5
MVO	-	1.5
Campul GMO (Capital City Products (mono and di-glycerides)	0.5	-
Lecithin	0.5	0.5
Artificial butter flavor	0.05	0.05
Beta-carotene solution (1%)	0.05	0.05
Water Phase		
Water	17	17
Morton salt	2	2
Chris Hansen starter distillate 15x	0.01	0.01
Alex Fries artificial cream flavor	0.01	0.01

Preparation Procedure

1. The oil phase was warmed to about 55°C.
2. The water phase was warmed to about 55°C.
3. The two phases were blended for 2 minutes in a Waring blender.
4. The resulting emulsion was cooled in a Kitchen Aid (placed in an ice-water bath) by mixing at speed 6 for 5 minutes.
5. The emulsion was soldified by placing at 4°C for 18 hours.

40% Fat Margarine

Ingredients	Percent (by weight)	
	1	2
Oil Phase		
Margarine Tub Oil (Cargill 270)	39	37.1
MVO	-	2.9
Campul GMO	1	-
Lecithin	0.2	0.2
Artificial butter flavor	0.05	0.05
Beta-carotene solution (1%)	0.05	0.05
Water Phase		
Water	58	58
Morton salt	1	1
Chris Hansen starter distillate 15x	0.01	0.01
Alex Fries artificial cream flavor	0.01	0.01

Preparation Procedure

1. The oil phase was warmed to about 55°C.
2. The water phase was warmed to about 55°C.
3. The emulsion was prepared in a Kitchen Aid mixer at speed 10, by the slow addition of the water phase to the oil phase. The addition took about 10 minutes. The mixing was continued for an additional 5 minutes.
4. The emulsion was blended in a Waring blender for 1 minute.
5. The emulsion was cooled in a Kitchen Aid (placed in an ice-water bath) by mixing at speed 6 for 5 minutes.
6. The emulsion was solidified by placing at 4°C for 18 hours.

Performance of Modified Canola Oil (MCO) in Tub Margarine

MCO was used as an ingredient in 60% fat tub margarine formulations. The following formulations were used:

Ingredient	Percent			
	1	2	3	4
Oil Phase				
Canola oil	50	-	50	-
Enzyme modified canola oil	-	60	-	60
Campul GMO (Capital City Products) (50% mono and 50% di-glycerides)	10	-	10	-
Lecithin	0.3	0.3	0.3	0.3
Artificial butter flavor (Givaudan)	0.05	0.05	0.05	0.05
Beta-carotene solution (1%)	0.05	0.05	0.05	0.05
Water Phase				
Water	38	38	38	38
Xanthan gum	-	-	0.2	0.3
Morton salt	1	1	1	1
Chris Hansen starter distillate 15x	0.01	0.01	0.01	0.01
Alex Fries artificial cream flavor	0.01	0.01	0.01	0.01

Preparation

1. The oil phase was warmed to about 55°C.
2. The water phase was warmed to about 85°C for 30 minutes, then cooled to 55°C.
3. Both phases were blended for 2 minutes in a Waring blender.
4. The emulsion was allowed to solidify by placing at 4°C for 18 hours.

Evaluation of Modified Peanut Oil (MPO) in Peanut Butter

To demonstrate the performance of MPO, partly defatted peanuts (32% oil) were roasted and ground. To the ground peanuts, peanut oil (used as a control) or MPO was added to bring the oil content to 50%. The mass was thoroughly mixed, passed through the grinder several times and packed in jars. The jars were stored at 37°C to accelerate oil separation.

Amberlite XAD-7 Preparation

One hundred and fifty grams of Amberlite XAD-7 placed into a 3 L glass funnel and thoroughly washed with 2 L of 0.1 M KCl, 2 L distilled water, 2 L 90% ethanol, 2 L distilled water and 2 L 0.1 M phosphate buffer pH 7.7. The beads were then dried on the filter for 1 hour.

Immobilization of Lipase from Pseudomonas Fluorescence

Three grams of Pseudomonas fluorescence lipase were dissolved in 100 mL 0.1 M phosphate buffer (pH 7.7) and 30 grams of washed Amberlite (wet weight) were added. The resulting suspension was placed into a 250 mL plastic flask and stirred at 4°C for 20 hr. The suspension was filtered, washed twice with 100 ml 0.05 M phosphate buffer (pH 7.7) and air-dried for 1 hour.

Immobilization of Lipases from Rhizopus Niveus

One and one half gram of washed Amberlite XAD-7

were rinsed with water and added into 10 mL of 50 mg/mL solution of the lipases in 20 mM $CaCl_2$. The pH was then adjusted to 7.5 with 1 M KOH and the suspension was placed on a rotor evaporator and dried under vaccuum at 30°C.

Immobilization of Lipase From Aspergillus Niger (Method 1)

Three hundred milligrams of lipase from Aspergillus Niger were dissolved in 16 mL of 20 mM $CaCl_2$ solution. To this solution, one gram (dry weight) of washed and dried Amberlite XAD-7 was added. The suspension was cooled to 0°C, stirred, and the enzyme was precipitated with 20 mL of acetone. The slurry was stirred for another 1 hour, centrifuged, and washed twice with 40 mL acetone. The immobilized enzyme was then dried under vacuum.

Immobilization of Lipase From Aspergillus Niger (Method 2)

One hundred milligrams of lipase from Aspergillus niger were dissolved in 1.5 mL of 20 mM $CaCl_2$ solution and 2 g Amberlite XAD-7 was added. The suspension was stirred for 2 hours and then placed on a rotor evaporator and dried under vacuum at 30°C.

EXAMPLES

Example 1

Ten grams (wet weight) of immobilized Pseudomonas fluorescence lipase was added to 500 grams of

peanut oil containing 16.7 mL water and stirred for 6 hours at 30°C to form a suspension. The reaction was stopped by removing the enzyme by centrifugation. Free fatty acids which are the reaction by-products were then removed by adsorption as follows: 310 grams of BioRad AG 1x8 ion exchange resin was added to the product of enzymatic transformation and the mixture was stirred for 15 hours, followed by the removal of the ion exchange resin by filtration. The composition of the final product was analyzed by gas chromatography. The results showed that the oil product contained the mixture of mono-, di-, and triglycerides in a weight ratio of 17.3:43.0:38.7.

The emulsifying properties of MPO were determined and compared with that of peanut oil. The emulsifying properties were characterized by the rate of oil/water separation as described in "Methods". The results, shown in Table 1, show that the emulsified product was significantly more stable than the emulsion formed with peanut oil.

Table 1

The Amount of Oil Separated from Water as a Function of Time

Time (days)	Peanut Oil(cc)	MPO (cc)
1	1.0	none
4	2.0	none
5	2.5	none
10	total	0.5-1.0*

*Large error is due to cream formation

Performance in Peanut Butter

Samples of peanut butter were prepared using the modified oil as described in "Methods", incubated at 37°C for 12 days and analyzed. Peanut butter to which regular peanut oil was added was very soft (too soft to spread), flowable, and showed definite signs of oil separation. Peanut butter to which MPO was added was thicker, spreadable, and showed no sign of oil separation.

Example 2

Four hundred grams of vegetable oil were partially hydrolyzed according to the procedure outlined in Example 1. A mixture of mono-, di-, and triglycerides in a weight ratio of 15.0:42.0:43.0 was formed. This modified vegetable oil (MVO) was evaluated as an ingredient in a yellow cake formula set out in the "Methods" section. MVO was used to replace partially hydrogenated oil and liquid vegetable oil in the formula. Cakes were formulated and prepared according to the above recipe.

Cake I contained 12% of partially hydrogenated vegetable oil. In cake II, this oil was replaced with liquid vegetable oil. In cake III the oil was replaced by MVO. Cake I was highly acceptable having good texture and crumb. Cake two was sticky, moist, and dense. Cake III was moist, yet crumbly with good but dense crumb. It was closer to Cake I than Cake II.

Example 3

MVO prepared as described in Example 2 was further evaluated as an ingredient in 80% fat and 40% fat tub margarines. The margarines were formulated and prepared as described in the "Methods" section. In the 80% fat formulations, Margarine 1 contained 0.5% mono- and di-glycerides. In Margarine 2, the emulsifier was replaced by 1.5% MVO. Both the margarines were very stable water-in-oil (w/o) emulsions and were comparable in quality. In the 40% fat formulations, Margarine 1 contained 1% mono- and di-glycerides, which was replaced by 2.9% MVO in Margarine 2. The margarines were stable w/o emulsions that were comparable in quality.

Example 4

Nine grams (wet weight) of immobilized Pseudomonas fluorescence lipase was added to 560 g of canola oil containing 25 ml of 20 mM $CaCl_2$ solution and the resulting suspension was stirred for 15 hours at 30°C. The reaction was stopped by removing the enzyme by centrifugation. Free fatty acids were then removed by adsorption as described in Example 1. The oil product contained the mixture of mono-, di-, and triglycerides in a weight ratio of 10.0:38.0:52.0. This modified canola oil was evaluated as an ingredient in 60% fat margarine. The margarines were formulated and prepared as described in the "Methods" section. The control (sample 1) did not form a stable product. The

emulsion broke before the product was cooled. The margarine prepared with the enzyme modified canola oil (sample 2) formed a stable very soft and pourable product that was homogeneous and did not break on cooling. Upon storage at room temperature for 18 hours there was some oil leakage. The addition of xanthan gum (samples 3 and 4) improved the quality of the products. Sample 3 formed a stable emulsion that broke on cooling, an improvement over the control (sample 1). Addition of xanthan gum to the margarine containing enzyme modified canola oil gave a stable, soft, emulsion with less oil leakage than sample 2.

Example 5

Three hundred milligrams (dry weight) of Aspergillus niger lipase immobilized according to Method 1 above, was added to 50 mL vegetable oil containing 4% of a 20 mM aqueous $CaCl_2$ solution, and stirred to form a suspension. The suspension was stirred vigorously at 37°C. After 6 hours, the reaction was stopped and the products analyzed by gas chromatography. They contained free fatty acids and a mixture of mono-, di-, and triglycerides in a weight ratio of 6.5:35.2:58.3.

Example 6

Eight hundred milligrams (dry weight) of Aspergillus niger lipase, immobilized according to Method 2 above, was added to 50 mL vegetable oil containing 4% of a 20 mM aqueous $CaCl_2$ solution and

stirred to form a suspension. The suspension was stirred vigorously at 37°C. Samples were removed periodically, and the composition of the reaction mixture was analyzed by gas chromatography. After 18 hours, the reaction medium contained free fatty acids and a mixture of mono-, di-, and triglycerides in a weight ratio of 6.0:3.50:59.0.

Example 7

One gram (dry weight) of immobilized lipase from Rhizopus niveus was added to 50 mL vegetable oil containing 4% of a 20 mM aqueous $CaCl_2$ solution and stirred to form a suspension. The suspension was stirred vigorously at 37°C. Samples were removed periodically, and the composition of the reaction mixture was analyzed by gas chromatography. After 5 hours the reaction medium contained free fatty acids and a mixture of mono-, di-, and triglycerides in a weight ratio of 8.1:33.0:58.9.

Example 8

Two grams (dry weight) of immobilized lipase from Pseudomonas fluorescence were added to 100 g of cocoa butter containing 4% (by weight) of a 20 mM aqueous $CaCl_2$ solution. The suspension was stirred vigorously at 37°C for 15 hours. Periodically samples were removed and the composition of the reaction mixture was analyzed by gas chromatography. The reaction was stopped by removing the enzyme by centrifugation. Free fatty acids were then removed by adsorption on a BioRad anion exchanger at 37°C as described in Example 1. The modified oil product

contained the mixture of mono-, di-, and triglycerides in a weight ratio of 20.0:37.0:43.0. Melting characteristics of the product were then determined. Oil samples were incubated at different temperatures and the physical state of oil was recorded. It was found that while cocoa butter melts at temperatures below 31°C, modified cocoa butter remains solid at as high as 36°C. By varying the degree of modification the whole spectrum of products with various melting characteristics can be obtained.

Example 9

One hundred and fifty milligrams (wet weight) of immobilized Pseudomonas fluorescence lipase were added to 10 ml of canola oil containing 7% (by weight) ethanol. The suspension was stirred vigorously at 37°C. Periodically samples were removed and the composition of the reaction mixture was analyzed by gas chromatography. After 14 hours the reaction medium contained fatty acid esters as a major by-product, and a mixture of mono-, di-, and triglycerides in a weight ratio of 11.0:35.0:43.0. The addition of ethanol instead of water to the reaction medium has a number of advantages. A higher concentration of monoglycerides can be achieved without glycerol formation. Since ethanol is soluble in most oils, the reaction medium forms a true solution which is easier to handle than an emulsion. The reduction of the amount of water in the reaction medium leads to the stabilization of

the enzyme. The reaction is easier to control and more valuable fatty acid esters are produced as by-products.

Equivalents

Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention describe specifically herein. Such equivalents are intended to be encompassed in the scope of the following claims.

CLAIMS

1. A method of producing a modified oil comprising the steps of:
   a. combining a fat or an oil, water or an aqueous solution and a selected lipase under conditions sufficient to form a suspension;
   b. agitating the suspension until partial hydrolysis of the fat or oil occurs;
   c. removing the lipase; and
   d. separating fatty acid by-products from the suspension.

2. A method of Claim 1 wherein the fat or oil is selected from the group consisting of: canola oil, soybean oil, sunflower oil, corn oil, olive oil, peanut oil, safflower oil, hydrogenated vegetable oil, butter fat, and cocoa butter.

3. A method of Claim 1 wherein the amount of water or aqueous solution is from about 2 to about 8% by weight.

4. A method of Claim 4 wherein the aqueous solution is 20 mM $CaCl_2$.

5. A method of Claim 1 wherein the lipase is selected from the group consisting of: porcine pancreatic lipase, Pseudomonas fluorescence

lipase, Aspergillus niger lipase, Mucor meihei lipase and Rhizopus niveus lipase.

6. A method of Claim 5 wherein the lipase is immobilized on a solid support.

7. A method of Claim 1 wherein the modified oil is a mixture of mono-, di- and triglycerides.

8. A method of Claim 7 wherein the amount of monoglycerides in the mixture is from about 1% to about 50%.

9. A modified oil produced by the method of Claim 1.

10. A modified oil produced by the method of Claim 2.

11. A food product containing the modified oil of Claim 1.

12. A modified oil comprising a glycerol-free, fatty acid-free mixture of mono-, di- and triglycerides.

13. A modified oil of Claim 12 wherein the amount of monoglycerides is from about 1% to about 50%.

14. A modified oil of Claim 12 produced from a fat or an oil selected from the group consisting of: canola oil, soybean oil, sunflower oil, corn oil, olive oil, peanut oil, safflower oil and cocoa butter.

15. A method of producing a modified oil comprising the steps of:

a. combining a fat or an oil, an alcohol and a selected lipase under conditions sufficient to form a suspension;

b. agitating the suspension until partial transesterification of the fat or oil occurs;

c. removing the lipase; and

d. separating fatty acid ester by-products from the suspension.

16. A method of Claim 15 wherein the fat or oil is selected from the group consisting of: canola oil, soybean oil, sunflower oil, olive oil, peanut oil, safflower oil, hydrogenated vegetable oil, butter fat and cocoa buffer.

17. A method of Claim 15 wherein the alcohol is selected from the group consisting of ethanol, propanol and butanol.

18. A method of Claim 17 wherein the amount of alcohol is from about 2 to about 20% by weight.

19. A method of Claidm 15 wherein the lipase is selected from the group consisting of: porcine pancreatic lipase, Pseudomonas fluorescence lipase, Aspergillus niger lipase, Mucor meihei lipase and Rhizopus niveus lipase.

20. A method of Claim 15 wherein the modified oil is a mixture of mono-, di- and triglycerides.

21. A modified oil produced by the method of Claim 15.

22. A food product containing the modified oil of Claim 15.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 90/06495

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC5: C 12 P 7/64, A 23 L 1/035, A 23 D 9/00

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
IPC5	C 12 P; A 23 L; A 23 D

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category *	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
P,X	US, A, 4906490 (ABRAHAM I. BAKAL ET AL.) 6 March 1990, see esp. column 2, l. 27-38 --	1-22
X	EP, A1, 0126416 (ASAHI DENKA KOGYO KABUSHIKI KAISHA) 28 November 1984, see esp. p. 7, l. 19-26 and p. 34, table 2 --	1-11,15-22
X	EP, A1, 0232933 (AKZO N.V.) 19 August 1987, see esp. p. 2, l. 25-49 --	1-11

* Special categories of cited documents:[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
30th January 1991	22. 02. 91
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	Alfred Prein

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
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X	US, A, 4861716 (ALASDAIR R. MACRAE ET AL.) 29 August 1989, see esp. column 1-2	1-11
X	US, A, 4863860 (PETER J. HALLING ET AL.) 5 September 1989, see the whole document	1-6,9-11
Y		15-22
Y	Chemical Abstracts, volume 98, no. 23, 6 June 1983, (Columbus, Ohio, US), Ohnishi, Kunio: "Studies on lipids of miso during aging. II. Effect of ethanol on changes of lipids during miso fermentation ", see page 508, abstract 196575g, & Nippon Shokuhin Kogyo Gakkaishi 1983, 30(2), 88- 93	15-22
X	Patent Abstracts of Japan, Vol 7, No 11, C145, abstract of JP 57-170193, publ 1982-10-20 MEITOU SANGYO K.K.	1-6
X	US, A, 31754 (DONALD E. MILLER ET AL.) 4 December 1984, see esp. column 3-4	12-14
X	Chemical Abstracts, volume 100, no. 5, 30 January 1984, (Columbus, Ohio, US), Kanisawa, Tsuneyoshi: "Production of flavors by biochemical methods. II. Production of ethyl ester mixture from butterfat by Candida cylindracea lipase ", see page 360, abstract 33406y, & Nippon Shokuhin Kogyo Gakkaishi 1983, 30(10), 572- 578	15-22

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

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X	Patent Abstracts of Japan, Vol 9, No 211, C300, abstract of JP 60- 78587, publ 1985-05-04 RAION K.K. --	15-22
X	EP, A2, 0274798 (UNILEVER NV) 20 July 1988, see esp. p. 2, l. 12-35 --	15-22

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. PCT/US 90/06495

SA 41953

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 28/12/90
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		JP-A-	1095740	13/04/89
		US-A-	4844921	04/07/89
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		US-A-	4956286	11/09/90

For more details about this annex : see Official Journal of the European patent Office, No. 12/82

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